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Heritable osteoarthritis. Diagnosis and possible modes of cell and gene therapy

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Our goals for osteoarthritis (OH) are straightforward: we need to define the causes of the disease and/or 'the cascade of events' that produce the degeneration of joint cartilage. From there we need to move on to methods of preventing or curing the disease.

As is well recognized, there are multiple causes for the degeneration of joint cartilage seen in OA. One of the hypotheses that we have pursued is that genetic mutations produce OA in a subset of patients with early onset forms of the disease. Another hypothesis we have pursued is that genetically caused OA may be the easiest target for both diagnosis and therapies for the disease.

Accordingly, we began several years ago to use the candidate gene strategy to search for mutations in the gene for type II procollagen (COL2A1) in patients with early onset OA. In a proband referred to us by Dr Roland Moskowitz and his colleagues at Case Western Reserve, we located a single-base substitution that converted a codon for arginine to a codon for cysteine at the α1-519 position in the triple helix of the protein.[1] The detection of the cysteine substitution suggested that the mutation was a disease-causing change because cysteine is not found in the interior of the triple helix of any fibrillar collagen. However, in common diseases such as OA it is frequently very difficult to be certain that a change seen in a gene does in fact cause the disease. Fortunately, Dr Moskowitz and his colleagues were able to provide us with data on the patient's family, and it was possible to show that the mutation was co-inherited with the disease in a statistically convincing manner (lod score of greater than 3.0). Eyre *et al.*[2] demonstrated that type II collagen extracted from the patient's cartilage formed abnormal disulfide bonds. Subsequently, we were able to demonstrate that the mutation generates abnormal fibrils in a purified system for assembly of type II collagen fibrils.[3]

Examination of the fibrils by atomic force microscopy demonstrated that the mutated protein has the unusual effect of increasing the depth of the gap region in collagen fibrils (E. Adachi, O. Katsuma, S. Yamashina, D. J. Prockop, and A. Fertala, submitted for publication). More recently, we developed a line of transgenic mice expressing the mutated gene. Our preliminary observations on the transgenic mice suggest that they develop cartilage changes that resemble OA (S.-W. Li, M. Arita, L. Ala-Kokko, D. J. Prockop, in preparation). Therefore, we now have thoroughly convincing data that the mutation in this family caused the clinical syndrome of early onset OA associated with evidence of a mild chondrodysplasia. Over the past several years, four other patients and families with similar findings have also been shown to have the same cysteine for arginine substitution at α1-519.[4] Three of the patients and families may have been related through an early Icelandic founder.

Our initial survey of some 50 patients with OA indicated that only about 2% of such patients have mutations in the gene for type II procollagen.[5] Therefore, Dr Leena Ala-Kokko, in our Center for Gene Therapy, has developed a strategy in which DNA from such patients is examined for the presence of mutations in six different collagen genes that are found in cartilage. This strategy is an ambitious one because collagen genes are large and have complex structures that include 50 or more exons. Therefore, complete analysis of such genes for the presence of mutations still presents serious technical problems. Our work has been aided by two developments. One development is a procedure referred to as 'conformation sensitive gel electrophoresis' (CSGE) developed by Dr Arupa Ganguly in our laboratory.[6] CSGE is a modification of previously proposed techniques for detection of single-base changes in PCR products by gel electrophoresis under appropriate conditions. We have extensively tested CSGE over the last several years and convinced ourselves that it detects 90% or more of single-base changes in PCR products.[7]

The technique is simple, rapid, and greatly reduces the amount of DNA sequencing we need to perform in order to analyze genes for mutations. The second development are the impressive achievements of Dr Leena Ala-Kokko and her graduate students in our Center and at the University of Oulu in defining the structures of a series of large and complex human collagen genes (see [8]). With these developments, we are perfecting protocols whereby we can analyze DNA from patients first for mutations in the COL2A1 gene, then in the three genes for type IX collagen (COL9A1, COL9A2, and COL9A3), and then in the two genes for type Xl collagen (COL11A1, and COL11A2). If no mutations are found, the same samples can be screened for mutations in other genes expressed in cartilage such as COMP, link protein and aggrecan.

The efforts in pursuing this expanded candidate gene strategy in OA are still incomplete. Our present estimate is that mutations in the six collagen genes will be found in about 5% of patients with early onset OA. One of our conclusions is that heritable OA is one end of a spectrum of cartilage disorders that range from severe chondrodysplasias that can prove lethal *in utero* to much milder

disorders of cartilage that appear only after many years of wear and tear in the joints.[9] In addition, we are convinced that a number of patients who present with early onset OA in adulthood probably had signs and symptoms of a mild chondrodysplasia in childhood, but those signs and symptoms frequently escape detection. Another conclusion is that the signs and symptoms seen in a patient are in some instances highly informative as to which gene contains a causative mutation, but in many instances, the same clinical syndrome can be produced by mutations in very different genes. For example, Dr Ala-Kokko and her associates have recently demonstrated that an RNA splicing mutation in one of the genes for type XI collagen (COL11A2) causes what appears to be a relatively common form of early onset OA (S. Annunen *et al.*, submitted for publication). As another example, a mutation in one of the three genes for type IX collagen (COL9A1) in two families and several unrelated patients causes marked degeneration intervertebral discs (S. Annunen *et al.*, submitted for publication).

Even though our knowledge about the causes of OA is still incomplete, preliminary attempts are being made in a number of laboratories to develop cell and gene therapies for the disease. One approach has been to isolate samples of normal cartilage from a patient, expand chondrocytes in the laboratory, and then use the expanded chondrocytes to replate the degenerated joint surface. One commercial concern is pursuing this strategy at the present time. A second approach has been to use viral vectors with which to introduce potentially therapeutic genes and proteins into joints. The appeal of this strategy is that the modified virus can probably be injected directly into joint spaces and thereby minimize any systemic effects. The use of viruses for gene therapy has proven to be more complex than initially assumed, but a large number of talented investigators are pursuing this strategy and it is likely to be fruitful in the near future. A third strategy has been to use as vectors for cell and gene therapy a subset of cells from bone marrow that have been referred to as mesenchymal stem cells or marrow stromal cells (MSCs). MSCs have attracted attention since the work of Friedenstein in the 1970s[10] in which he demonstrated that bone marrow contains a subset of stem-like cells that are distinct from stem cells for hematopoietic precursors and that serve as precursors of non-hematopoietic cells such as osteoblasts, chondrocytes and adipocytes. Friedenstein's work has now been amply confirmed and extended by a large number of investigators including Piersma in the Netherlands, Owen at Oxford and Caplan at Case Western Reserve [for review, see [11]].

In our laboratory we carried out a series of experiments in which MSCs were isolated from transgenic mice containing a marker collagen gene and infused systemically into X-ray irradiated isogenic mice.[12] The results demonstrated that the donor cells were difficult to detect at one week, but they appeared in a variety of non-hematopoietic tissues at one month and five months. They accounted for between 2 and 20% of the cells in bone, cartilage, lung and several other tissues. In part based on our results in mice, Drs Ed Horwitz and Malcolm Brenner at St. Jude Children's Research Hospital in Memphis initiated a clinical trial in patients with severe osteogenesis imperfecta (OI).[13] The trial was based on the assumption that whole bone marrow may contain enough MSCs to engraft in bone and replace osteoblasts containing a mutated collagen gene with osteoblasts containing a normal gene. Three patients with severe OI who were marrow ablated and received bone marrow transplants from matched siblings have been evaluated six months after therapy.[14] All three patients have shown improvement in fracture rate, growth, bone histomorphometry and total body mineral as assayed by DEXA. However, osteoblasts from bone spicules obtained from two of the probands indicated that only 1 to 2% of the cells were of donor origin. Therefore, it is not clear whether the clinical improvement in the patients can be explained by infusion of MSCs or by some unexpected consequence of marrow ablation and bone marrow transplantation.

In more recent experiments,[15] we have asked the question of whether human MSCs will engraft and migrate like neural stem cells after infusion into the brains of rats. The background of the experiment was that a large body of data developed over the last five years or so has shown that the brain normally develops from stem-like cells that line the ventricles and then move along known pathways through the layers of the brain. As they reach a given layer, they acquire the phenotype of the cells in that layer. The process is prominent during early development of the brain, but it continues at a slower rate in adults. Our thinking for the experiment was also guided by the therapy for Parkinson's disease developed by a group of Swedish neurosurgeons in which cells isolated from the brains of human abortuses were injected into the basal ganglia of patients [see [15]]. Over 80 patients with Parkinson's disease have received the treatment, and a number have shown objective signs for improvement [see [16]]. The application of the treatment is limited, however, by the difficulty in obtaining the necessary human fetal cells. In our experiments, one surprise was that the human MSCs did not induce any inflammatory or immune reactions after injection into the brains of rats. Another surprise was that the cells did not aggregate and induce gliosis as is seen with infusion of fibroblasts and a number of other cell types. Instead, the cells migrated along known pathways for the migration of stem cells and integrated into multiple layers of the brain. Assay of sections of the brain indicated that at least 15% of the human cells had survived for 6 months.

These and other observations by other investigators suggest that MSCs are attractive vectors for cell and gene therapy in a variety of diseases. They are relatively easy to isolate from a patient and engineer in culture. Also, after systemic infusion, they home to many tissues and then apparently they acquire the phenotypes of the cells normally found in the same tissues. For example, they become chondrocytes in cartilage. The implications for therapy of OA is that it may be possible to isolate MSCs from a patient, engineer the cells so they express a therapeutic protein such as IL-Ra, IL4, or IL1O, and then use the cells for therapy of the same patient. The expression of the potentially therapeutic protein can be engineered so that it is driven by cartilage specific protein. The most likely application would be by intra-articular injection of the gene-engineered MSCs. However, it is possible that systemic infusion of the same cells may be followed by a sufficient number of the cells homing to cartilage and then expressing the therapeutic protein under the influence of the cartilage-specific promoter.

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